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Discussion

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Dr. Kass has reported highly reproducible measurements of the rate of clearance of staphylococci and other bacteria from the lungs of mice after aerosol inoculation. The aerosol particles were 1 to 3 μ in diameter, and the dose, given in a 30-min inhalation, was sufficiently large to permit recovery of at least 50,000 colony-forming units. Studies of lung sections with fluorescein-labeled antibody and by conventional staining methods revealed staphylococcal antigen and some intact bacteria in alveolar lining cells.

With this model, the effect of hypoxia, alcohol, starvation, and other influences was studied. In addition, it was shown that influenza virus infection interfered with the clearance of *Staphylococcus aureus* from the lung.

At this point, it is perhaps of interest to consider briefly the relationship of clearance of staphylococci by alveolar macrophages, referred to by Dr. Kass, with other clearance mechanisms. It is well appreciated at this conference that particles of the size used by Dr. Kass largely escape trapping in the nasopharynx and are carried to the lung. Here a large percentage are deposited, and the remainder are exhaled. Sites available for deposition are the alveoli, the alveolar ducts, respiratory bronchioles, and more proximal airway structures. Although gas exchange occurs quite readily between the tidal air and the alveoli through the layer of residual air in the alveoli, this is effected chiefly by the process of molecular diffusion. In contrast, only 10 to 20% of aerosol in tidal air actually exchanges with residual air with each breath, and molecular diffusion is not a significant factor with particles of the size presently under discussion. It is suggested, therefore, that substantial alveolar penetration will require prolonged periods of breathing of aerosol, probably of the order of that used by Dr. Kass. With a few breaths, particles may be deposited in the lower respiratory tract proximal to the alveoli, and, with further breathing, the site of major deposition will progress peripherally, ultimately to the alveoli, as alveolar wash-in is completed. Parenthetically, I wonder if the slow movement of particles from tidal air to residual air may not be an important means of protection against toxic or infectious particulates in the environmental air.

Once deposited, particles may be removed from alveoli by alveolar macrophages and carried into pulmonary lymphatics. Some macrophages filled with particulates may also be discharged up the airway to the muco-ciliary blanket and then carried up the trachea. In the case of microorganisms which deposit in the respiratory bronchioles, the mode of disposition is not clear. Alveolar macrophages are apparently not available here, and the muco-ciliary blanket begins more proximally. Some studies, however, have described a hyperreactivity of respiratory bronchiolar lining cells which may be a special means of protection in this area. The small volume of lung airway represented by the tracheobronchial tree appears to be the best protected. Inhaled particles which deposit here are carried rapidly up to the posterior pharynx by the muco-ciliary mechanism, where they may be expelled or swallowed.

At present, I know of no studies which adequately describe relative degrees of deposition of small particles in peripheral lung areas in relation to the duration of exposure to small-particle aerosol. I believe the question to be of importance, since, if the foregoing concept is correct, it would be possible to deposit small-particle aerosol in

sites other than the alveoli, and mechanisms other than alveolar macrophages would be called forth to clear them from the lung. Instances of this sort may regularly occur in the natural spread of airborne infection.

Evidence for significant deposition of small particles at sites other than the alveoli is found in the work of McGavran et al. (4), who observed that pulmonary lesions of psittacosis in monkeys, after small-particle aerosol inoculation, developed around foci in respiratory bronchioles and that none were found developing around alveoli. These findings cannot be considered proof, however, since lesions develop a considerable time after inoculation, and a number of factors could influence the site of development of infection during this period.

I was especially interested in Dr. Kass' report that clearance of staphylococci from the lung was impaired in the presence of influenza virus infection, but only after it had progressed for 6 to 8 days. As he suggested, this coincides in time with the occurrence of some human cases of bacterial pneumonia complicating influenza. Harford et al. (2) in 1948 showed a similar result with pneumococci. In their studies, instilled pneumococci multiplied rapidly in mice during the 5th to 6th day of viral influenza, leading to pneumonia and death. Gerone et al. (1) in 1957, in similar experiments, found a rapid increase in pneumococci in the lung and high mortality in mice given bacterial challenge 6 to 9 days after influenza virus PR8 inoculation, but bacterial challenges given before this time were without effect. Although Kass did not report on mortality, it seems likely that an appreciable occurrence of pneumonia and mortality might have resulted in his studies.

Apparently quite distinct from the foregoing was the observation by Janssen, Chappell, and Gerone (3) that guinea pigs given *S. aureus* at the time of inoculation with influenza virus showed a high mortality within 48 hr. Influenza virus or *Staphylococcus* alone in the same doses had no effect. The effect was shown to be dependent on live influenza virus, and it did not occur in animals with influenza antibody produced by prior challenge. On the other hand, killed staphylococci served as well as live cultures in causing death. Animals dying of this synergistic combination showed pulmonary consolidation. However, staphylococci could not be cultured from the lungs (in animals given live cultures), although influenza virus was present in high titer. One is tempted to compare these results with the occasional case of rapidly fatal human influenza in which no evidence of bacterial pneumonia is found. Thus, there may be two forms of interaction of staphylococci and influenza virus in animal infection which have a counterpart in natural human illness, i.e., bacterial superinfection late in the course of influenza (referred to by Kass), and an early, often fatal influenza, apparently unrelated to bacterial infection, but conceivably contributed to by constituents of killed staphylococci [referred to by Janssen et al. (3)] which have remained in the lung.

Dr. Kass' studies of metabolic and other factors which influence lung clearance are of great interest. If the relationship of lung clearance of microorganisms to the pathogenesis of pulmonary infection can be precisely defined, this model could serve an extremely useful purpose in attempts to identify mechanisms of susceptibility and resistance to infection. It would be highly desirable to extend some of these techniques to man, if the safety of the methods could be assured, but the problem is technically an imposing one.

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